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**UNPUBLISHED PRELIMINARY DATA**  
Detection of Extraterrestrial Life .

Method II: Optical Rotatory Dispersion

Final Report

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Submitted by  
Melpar, Inc..  
3000 Arlington Boulevard  
Falls Church, Virginia

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## 1. INTRODUCTION

This document is submitted as the Final Report on NASA Contract NASW-557. The object of this study was to develop polarimetric methods to determine the presence of optically active materials, particularly DNA (deoxyribonucleic acid) or its congeners, in soil suspension. Provided that such methods can be developed, the determination of the existence of life (as known terrestrially) on other planets may be accomplished by remote instrumentation. The Cotton region of the spectrum associated with optically active biological compounds is of principal interest here, because of the intensely high activity in the absorption bands of such materials.

To achieve this goal the program was designed to consist of two phases. One phase dealt with instrumental development and the other with methods of processing soil to achieve sufficient concentrations of optically active components.

Under the instrumental phase of this program, the Rouy electronic ratio (the difference to the sum) for measuring optical activity was first considered. However, measurements performed on the Rouy polarimeter were found to be qualitative rather than quantitative. Consequently two new experimental approaches for measuring optical activity were developed by Melpar. They are the electronic ratio of the difference to a constant, and a modified form -- the simple ratio. Based upon the new developments, an instrument has been designed to detect extraterrestrial life by measuring the optical activity in soils.

The second phase of this program has been concerned with the processing of soils. By investigating certain extraction procedures, it has been found

that it is possible to concentrate the organic components in soil. Based upon the above findings, an extraction procedure has been developed which will enable extraterrestrial polarimetric measurements to be made on these organic components.

## 2. INSTRUMENTAL DEVELOPMENT

### 2.1 Evaluation of the Rouy Electronic Ratio (the Difference to the Sum).

#### 2.1.1 Background

It is generally known that the polarimetric measurements of optically active biological materials at their absorption bands result in intensely high activity. Measurements in these regions are subject to several difficulties. These are, (1) circular dichroism, which can be associated with the unequal absorption of right and left circular-polarized light, (2) absorption of light due to chromophores, and (3) scattering. Theoretically, the Rouy electronic ratio, the difference to the sum, is capable of handling these obstacles. For details see Anal. Chem, 37, 594 (1961) and Anal. Chem. 35, 627 (1963).

#### 2.1.2 Results

An investigation of the performance of the Rouy-type polarimeter indicated that the light beams, polarized  $+0$  and  $-0$ , were of unequal intensity. It was found that the differences in intensities between the two beams could be attributed to the following:

a. The presence of reflecting surfaces which are located between the polarizer prisms and analyzer prisms result in the generation of elliptically polarized light. The amount of this light generated depends upon the angle with which the plane polarized beam makes with the reflecting surfaces. This elliptically polarized light can pass through the analyzer prism. Since the Rouy-type polarimeter contains two polarizing prisms, the positioning of the optic axis of these prisms with respect to the reflecting surfaces is important. If the optic axes of the polarizing prisms are at different angles with respect

to the reflecting surfaces, different amounts of elliptically polarized light will pass through the analyzer prism. To overcome this problem, it was necessary to reposition the polarizing prisms so that their optical axes make equal angles with the optical axes of these reflecting surfaces. On this basis, equal amounts of elliptically polarized light would be present in both beams. By using the ratio of the difference to the sum, this effect of elliptically polarized light will be cancelled.

b. The transmission of the Glan prisms decreases as a function of wavelength. Also the transmission of the Glan prisms at designated wavelengths cannot be matched. Consideration of this observation, in the theoretical development of the ratio of the "difference to the sum," indicated that the unequal transmission between the right and left polarizing prisms renders the measured ratio a complex function of the angular rotation.

### 2.1.3 Conclusions

Although it was possible to overcome the problem of elliptically polarized light being generated from a reflecting surface when using the Rouy-type polarimeter, the problem of obtaining matched Glan prisms with equal transmission at designated wavelengths could not be overcome. This is because the state of the art in cutting Glan prisms has not achieved the precision necessary for making optical rotatory measurements using this instrument. Thus, it was impossible to obtain quantitative measurements using the Rouy-type polarimeter.

## 2.2 Development of the Electronic Ratio (the Difference to a Constant).

### 2.2.1 Background

It had been pointed out in section 2.1.3 that the transmissions of the Glan prism could not be matched at designated wavelengths. Thus, it was



necessary to develop a polarimetric technique which would not be affected by this problem. In addition, it was necessary that this new approach would be capable of measuring optical activity in the absorption band of an optically active biological substance.

#### 2.2.2 Results

Upon completion of a mathematical analysis of the problems associated with optical-rotation measurements in the absorption band of an optically active substance, a new electronic ratio which may be defined as the ratio of the difference to a constant, was evolved. For a detailed development see the Second Quarterly Report to NASA, Contract No. NASW-557, 20 March to 19 June 1963. This ratio adequately takes into account circular dichroism and absorption effects which are encountered in obtaining optical rotation measurements in the absorption region of the spectrum.

This new experimental approach can readily be applied to both double-beam and single-beam instruments. To obtain optical rotatory dispersion measurements, Melpar has modified the Cary model 15 double-beam spectrophotometer. Figure 1 represents the polarizer sample holder and analyzer arrangement for insertion into the sample chamber of this spectrophotometer. Figure 2 represents the polarizer and sample holder for insertion into the reference chamber of this instrument. It should be noted in figure 2 that an analyzer prism is absent from the reference unit.

To obtain optical rotatory dispersion measurements on this instrument, an optically inactive substance such as water is placed in both the reference and sample units. The optical axis of the analyzer is now at an angle of  $+ \theta$  degrees, with respect to the optical axis of the polarizer. A series of

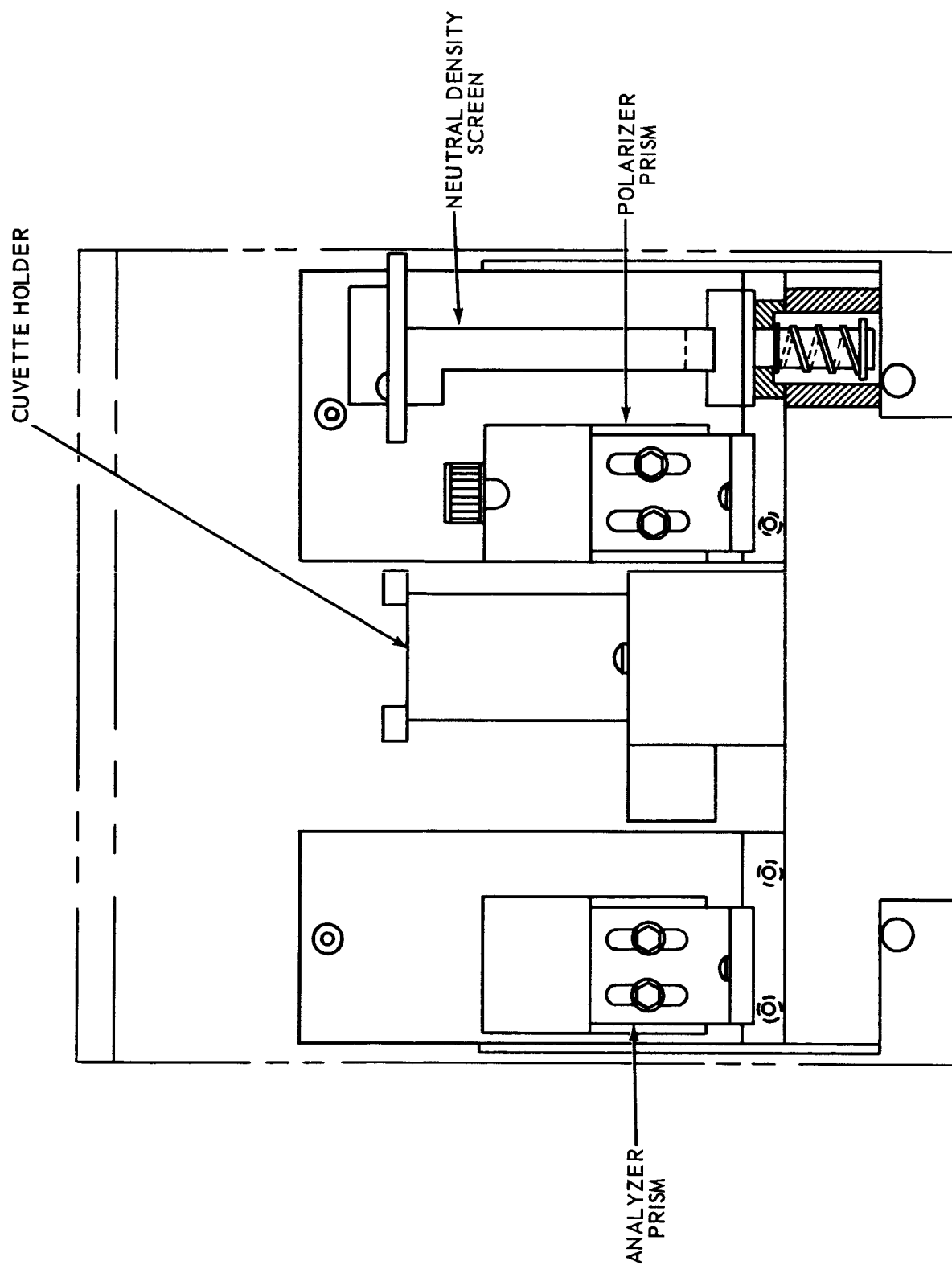


Figure 1. Sample Unit

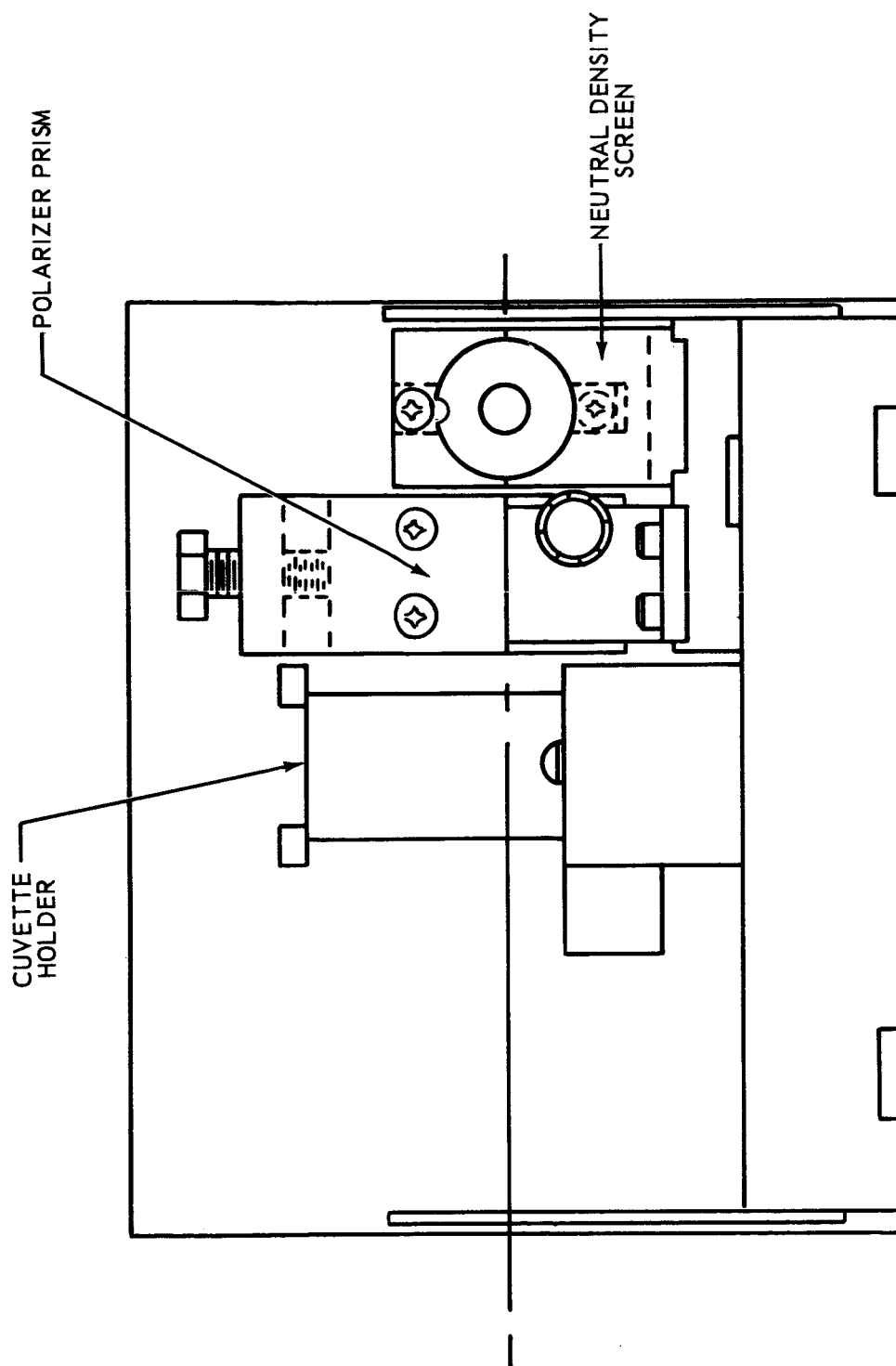


Figure 2. Reference Unit

transmission measurements are obtained as a function of wavelength. The water in both units is replaced by identical optically active samples and the transmission measurements are repeated. By dividing the transmission measurements obtained for the water into the transmission measurements obtained for the optically active samples, a ratio  $R_1$  may be obtained. By repeating this process, where the optical axis of the analyzer is now set at  $-9$  degrees with respect to the optical axis of the polarizer, a ratio  $R_2$  is obtained. Subtracting  $R_2$  from  $R_1$  now gives the ratio defined as the difference to a constant. This ratio is directly related to a linear polarimetric scale.

The unequal transmissions of the Glan prisms at designated wavelengths do not effect the measurement of optical activity activity when using the ratio of a difference to a constant. Since a ratio of the transmission of the optically active sample to water is determined, the unequal transmissions of the prisms cancel out. For a detailed treatment, see the Second Quarterly Report to NASA, Contract No. NASW-557, 20 March 1963 to 19 June 1963.

### 2.2.3 Conclusions

A comparison of the polarimetric scales related to the ratio of the difference to a constant, and the scale related to Rouy's ratio (the difference to the sum) show them to be similar when the angle  $\theta$  between the optical axis of the analyzer and polarizer is set at  $45$  degrees. However, by departing for this angular setting, the similarity of the polarimetric scales no longer exists. This is encountered when measurements are performed on optically active molecules in their absorption bands. The ratio of the difference to a constant still retains to linearity with respect to its polarimetric scale; whereas, the measured ratio of the difference to the sum becomes a complex

function in terms of its polarimetric scale. The advantage of performing polarimetric measurements at an angular setting greater than 45 degrees is the increase in the sensitivity of the measurements (Rouy, A. L. and Carroll, B. Anal. Chem., 33, 594 (1961)).

## 2.3 Evaluation of the Simple Ratio

### 2.3.1 Background

It is apparent from the discussion in section 2.2.2 that the use of the ratio (the difference to a constant) for the detection of optical activity on other planets by remote instrumentation would be difficult to perform. Complex electronic computations would be necessary to obtain this ratio. Therefore, a modified form of the ratio of a difference to a constant, the simple ratio, was considered. This ratio is defined as  $R_1$  (see section 2.2.2) which requires only one-half of the measurement necessary to obtain the ratio of the difference to a constant. To relate the simple ratio to a linear polarimetric scale, the angle between the optical axis of the analyzer and polarizer must be set at 45 degrees. For a detailed treatment see the Third Quarterly Report to NASA, Contract No. NASw-557, 19 June 1963 to 20 September 1963. The simple ratio is also not affected by the unequal transmission of the Glan prisms at designated wavelengths. In addition, it is capable of measuring optical activity in the absorption band of an optically active substance.

### 2.3.2 Results

#### 2.3.2.1 Evaluation of the Theoretical Development of the Simple Ratio:

To obtain optical rotatory dispersion measurements on the Cary Model 15 spectrophotometer using the simple ratio, water is placed in both the sample

and reference units. A series of transmission measurements are obtained as a function of wavelength. The water in both units is replaced by identical optically active samples, and the transmission measurements are repeated. By dividing the transmission measurements obtained for the water into the transmission measurements obtained for the optically active samples, the simple ratio,  $E_1/E$ , is obtained. This ratio is directly proportional to a linear polarimetric scale.

To test the validity of the theoretical considerations used in the development of the simple ratio, the optical activity of standard solutions such as sucrose, d-tartaric and l-tartaric acid were measured as a function of wavelength. The result of these measurements for two sucrose solutions, which were prepared to give a two degree and four degree rotation at the sodium D line, are shown in figure 3. The triangular points represent the angular rotations calculated from the specific rotations of sucrose obtained from the Handbook of Chemistry and Physics, 35th edition, Chemical Rubber Publishing Co., p. 2734. It may be seen from this figure that the angular rotations calculated from the literature are in agreement with those determined experimentally.

The polarimetric scale, which is proportional to the simple ratio, must be symmetrical about zero angular rotation. That is, if the angular rotation of two substances having the same angular rotation, but opposite in sign, are measured, their rotations must differ only in sign. The optical rotatory dispersion curves for 10% solutions of the d and l form of tartaric acid for the wavelength range from 350 to 250 mμ are shown in figure 4. From this figure, it may be seen that the angular rotation for both forms of tartaric acid are the same, but opposite in sign.

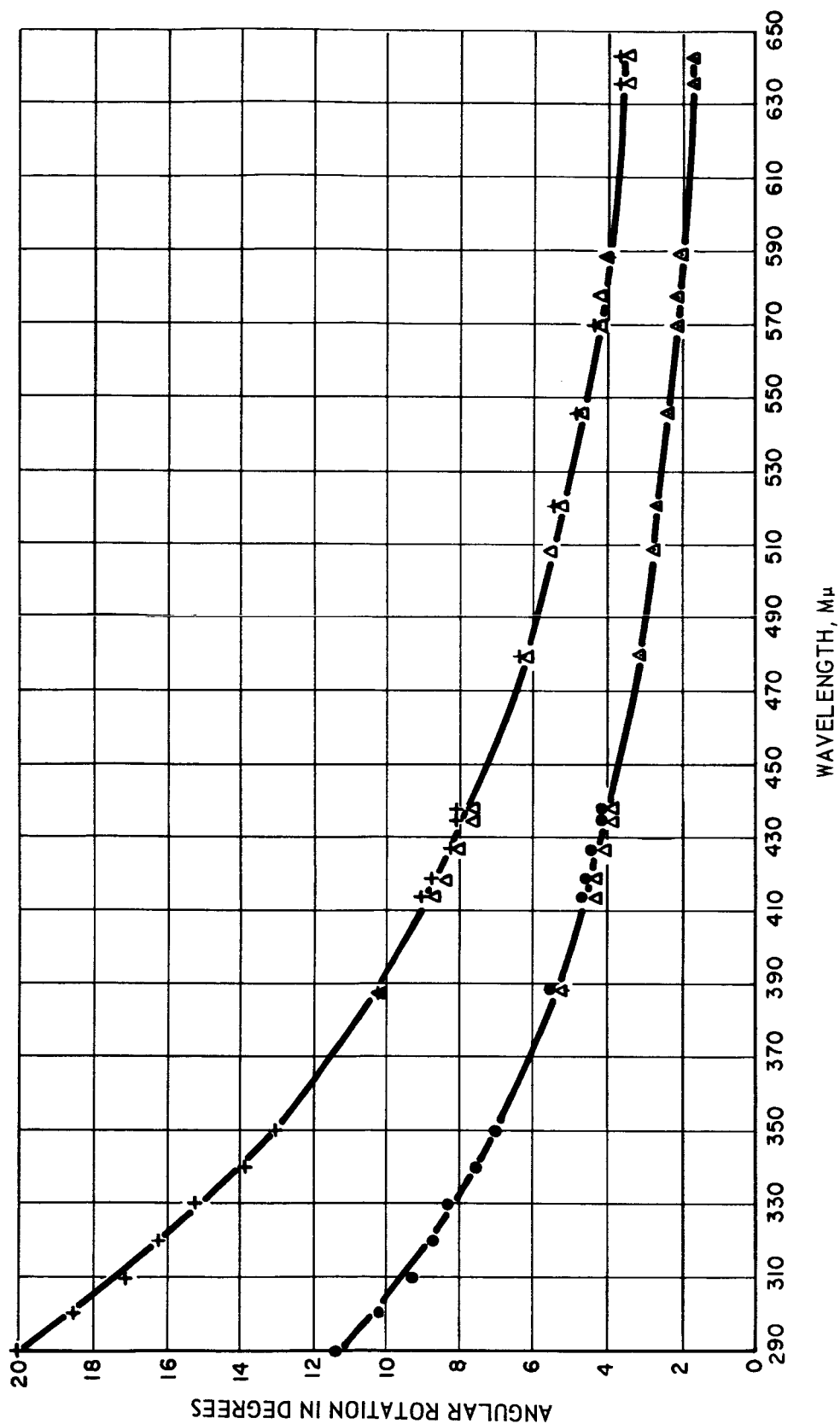


Figure 3. Optical Rotatory Dispersion Spectra of Sucrose: + - +, 4.01 Degrees at Sodium D Line; . - ., 2.01 Degrees at Sodium D Line; Δ - Δ, Literature Values

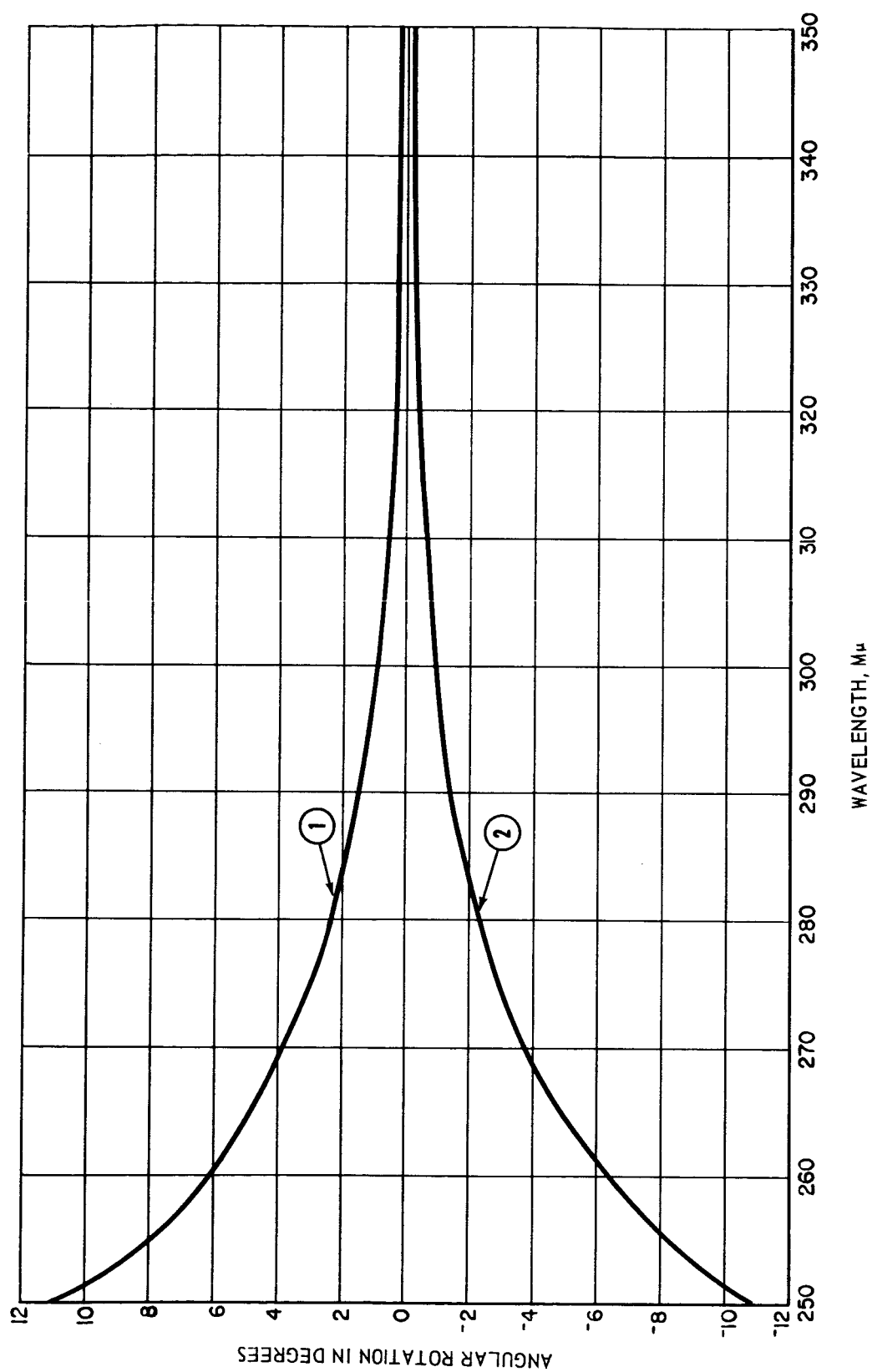


Figure 4. Optical Rotatory Dispersion Spectra of Tartaric Acid:  
(1) L-Tartaric Acid at 10% and (2) d-Tartaric Acid at 10%



Scattering of light by slightly turbid media does not affect the measurements of optical activity by using the simple ratio. For the experimental results validating this statement see the Third Quarterly Report to NASA, Contract No. NASw-557, 19 June to 20 September 1963.

2.3.2.2 Instrumental Design of a Flight-Prototype Polarimeter: Since the theoretical predictions based on the development of the simple ratio has been proven experimentally, a polarimeter based upon this development has been designed to detect extraterrestrial life remotely, by measuring the optical activity extracted from soils.

The optical system of this instrument, shown in figure 5, is a two-beam folded system designed to use the same sample for both the reference and the test beams. The important difference in the two paths is the presence of the analyzer prism in the test beam. In both beams, the light from the source is collimated by the first lens, polarized by the prisms, passed through the sample chamber, and imaged by the second lens to illuminate the sensitive surface of the detector.

The radiant energy source for this instrument can be a low-power mercury discharge tube approximately 4 mm in diameter and 40 mm long with a discharge length of 3 to 4 mm. Electrical power input to the lamp will be less than one-quarter watt.

The choice of this type of lamp is based on the high efficiency of low-pressure mercury lamps in the production of 2537 mμ energy, and on the fact that the organic materials to be detected have high specific rotations in the 280 to 245 mμ region.

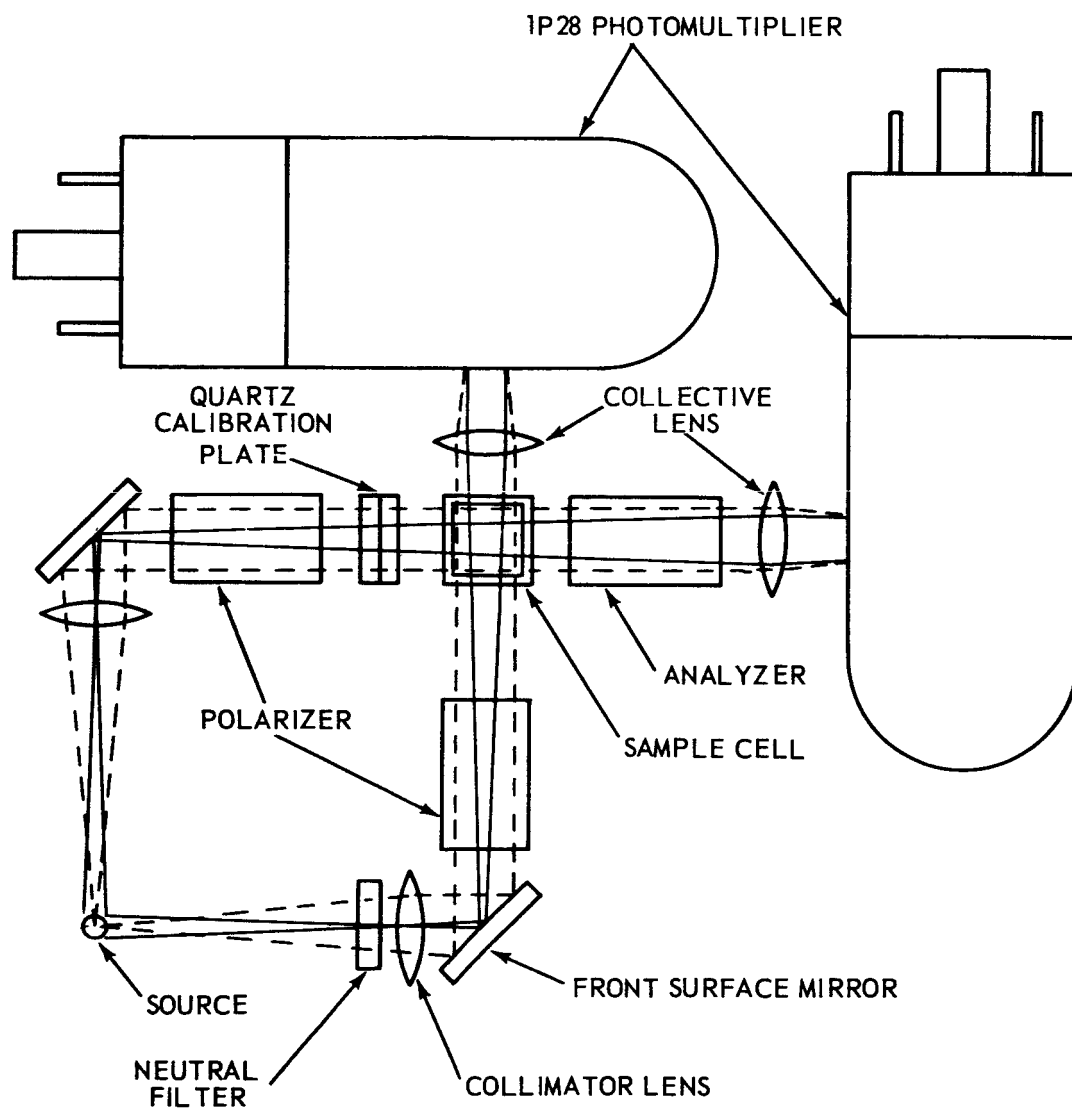


Figure 5. Optical System

The contents of the sample chamber will scatter and absorb some of the incident energy. If the sample rotates the plane of polarization, only that component of the incident beam which is parallel to the plane of the analyzer prism reaches the detector in the test beam. Rotation of the plane of polarization has no effect on the energy reaching the reference beam detector. Since the analyzer prism absorbs some energy, a filter is placed in the reference beam so that the energy reaching the two detectors is the same in the absence of an optically active sample. This matching of the beam energies at the detectors allows both detectors to be used in the same region of their response curve, which will minimize errors introduced by changes in sensitivity with signal level when this level is changed by absorption and scattering by the sample.

The two polarizing prisms and the analyzer will be identical prisms of an air-spaced type, giving a useful beam divergence of about 4 degrees full field. The clear aperture of the prisms will be about 8 mm. Air-spaced prisms forms are necessary, since the differential expansion of crystalline quartz and any known optical cement would probably crack the prisms when they are subjected to the large changes in temperatures expected during a space trip.

While the filter used to equalize the transmission of the two paths is shown as a separate element in the figure, it can probably be a partially-transmitting evaporated aluminum film on one of the lens surfaces.

The four lenses will be identical simple  $f/5$  elements of fused quartz, shaped to give minimum spherical aberration. Greater aperture is not practical with single-element spherical lenses, and the energy gain which could be achieved by using quartz-fluorite combinations or aspheric surfaces does not justify the increased complexity.

The detectors shown in the figure are 1P28 photomultiplier tubes which will definitely give a satisfactory signal-to-noise ratio, with the smallest obtainable mercury lamp.

The quartz test place, which is shown in the test beam, is to be used to provide a positive assurance that the complete instrument is functioning properly after it has landed extraterrestrially. This plate is a combination of a plate of right-handed quartz and one of left-handed quartz with the thicknesses chosen so that a net rotation of the plan of polarization, about 6 degrees is produced. The double plate construction is necessary to allow mechanically reasonable thicknesses since quartz produces a rotation of some 140 degrees per mm at 2537 angstroms. The quartz test plate is removed from the optical path by means of a squib actuator before the sample to be tested is placed in the sample chamber.

The components required to perform the necessary computations are described later in this section. The output current of the photomultiplier tubes are amplified to usable values to perform the computations. Figure 6 shows the overall electronic requirements. Two photomultiplier tubes are used to detect the presence of any light rotation. Initially a quartz crystal will be inserted without the sample. The amount of rotation of the plane of polarization is indicated by the ratio of the two photomultiplier signals. This ratio will be calculated and will be stored for further computations. The programmer will then remove the quartz crystal, and the ratio of the light in absence of any sample is again calculated to determine that the photomultiplier tube outputs are the same. This ratio is again stored. A third ratio is calculated with the sample programmed in place, and this ratio

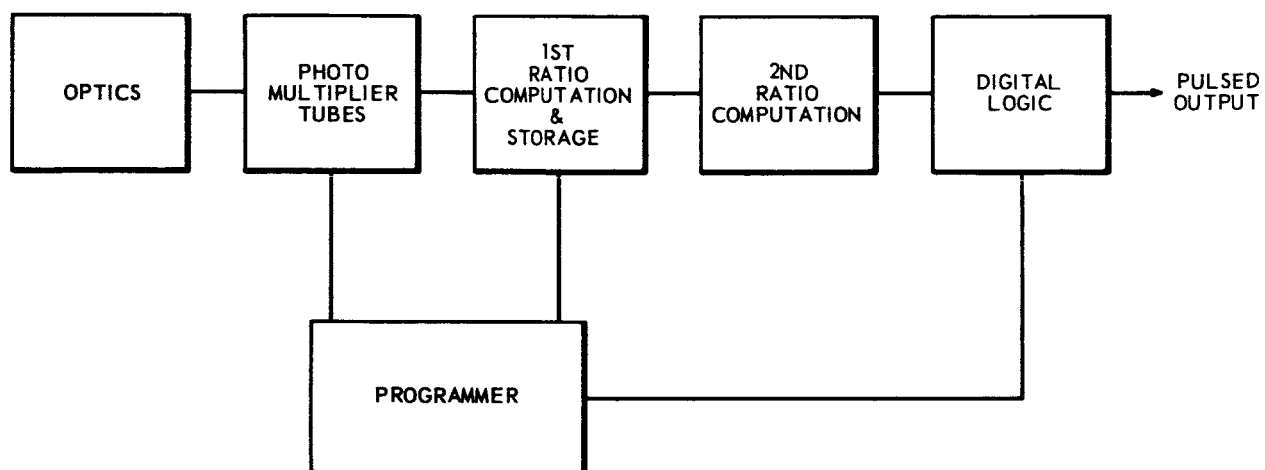


Figure 6. Block Diagram of Electronic Computation

is stored. All of the information necessary to determine if a rotation of the polarized light has occurred is now available. The remainder of the electronics required provides a pulsed output to indicate a "yes" or "no" answer. Therefore, simplification of the overall design would be accomplished if sufficient space is available in the telemetering channels to transmit the three stored ratios. Each ratio would require no more than 10 bits for three significant figures, and a total of 30 bits would be needed to transmit this information.

Assuming that this space is not available, one of the stored voltages is compared again in two steps with the other two stored voltages, and a pulsed output is derived so that no pulse represents "no measurement"; one pulse represents a valid "no" answer; and two pulses represent a valid "yes" answer.

The ratio computations to be performed rely on a Hall effect multiplier device and a high-gain differential amplifier. The Hall effect device employs a conducting material placed perpendicular to a magnetic field. A voltage is developed to be stored for subsequent computations. The programmer will provide the switching when the ratios are to be computed. The first voltage ratio is labeled  $K_c$  and is a measure of a fixed amount of rotation with a quartz crystal in position. The second ratio is noted a  $K_o$  and is really the reference ratio with the quartz removed and no sample in position. The third ratio,  $K_s$ , is ratio voltage attained with the sample in place. Once these ratios have been computed and stored, the initial phase of the test has been completed; and there only remains the need to determine the valid rotation of the polarized light with the sample in place as designated by  $K_s$ .

A capacitor and operational amplifier will be employed to perform the memory task when connected similarly to an integrator of a fast input time constant. The input and feedback impedances are then composed of resistances shunted by capacitors of such values, that the time constant of each network is the same. In this way, the amplifier acts as an inverting amplifier and can be connected to the output circuit of the ratio computer through the proper switching. At the storage instant, the resistance in the feedback impedance is disconnected, and the impedance is replaced with an equivalent resistance to ground at amplifier input. Under this condition, the input signal is zero, and the stored voltage is retained for further computation.

A second ratio computation will be performed at two intervals. This computation is necessary to eliminate some of the variable factors derived in the first computation. The computation is the same as previously described and is shown in figure 7. The programmer will select the stored ratio  $K_c$  and  $K_o$ , and a new ratio  $K_c/K_o$  is computed and then amplified for presentation to the digital logic functions. Figure 8 shows the manner in which the final output pulse is obtained. The flip-flop is initially preset by the start pulse from the programmer. The  $K_c/K_o$  ratio should properly be a value of 12.0 volts. A pulse is obtained across the material in a direction perpendicular to both the magnetic field and the current flowing through the conducting material. This voltage is called the Hall voltage. It arises from the deflection of the moving charge carriers toward the sides of a conducting element by the applied magnetic field, and it results in a transverse electrical field. The Hall voltage is proportional to the product of the magnetic field and the current passing through the element. The multiplication error in this device is 0.3%. It has a negative temperature coefficient of 0.1% per °C and can be easily compensated.

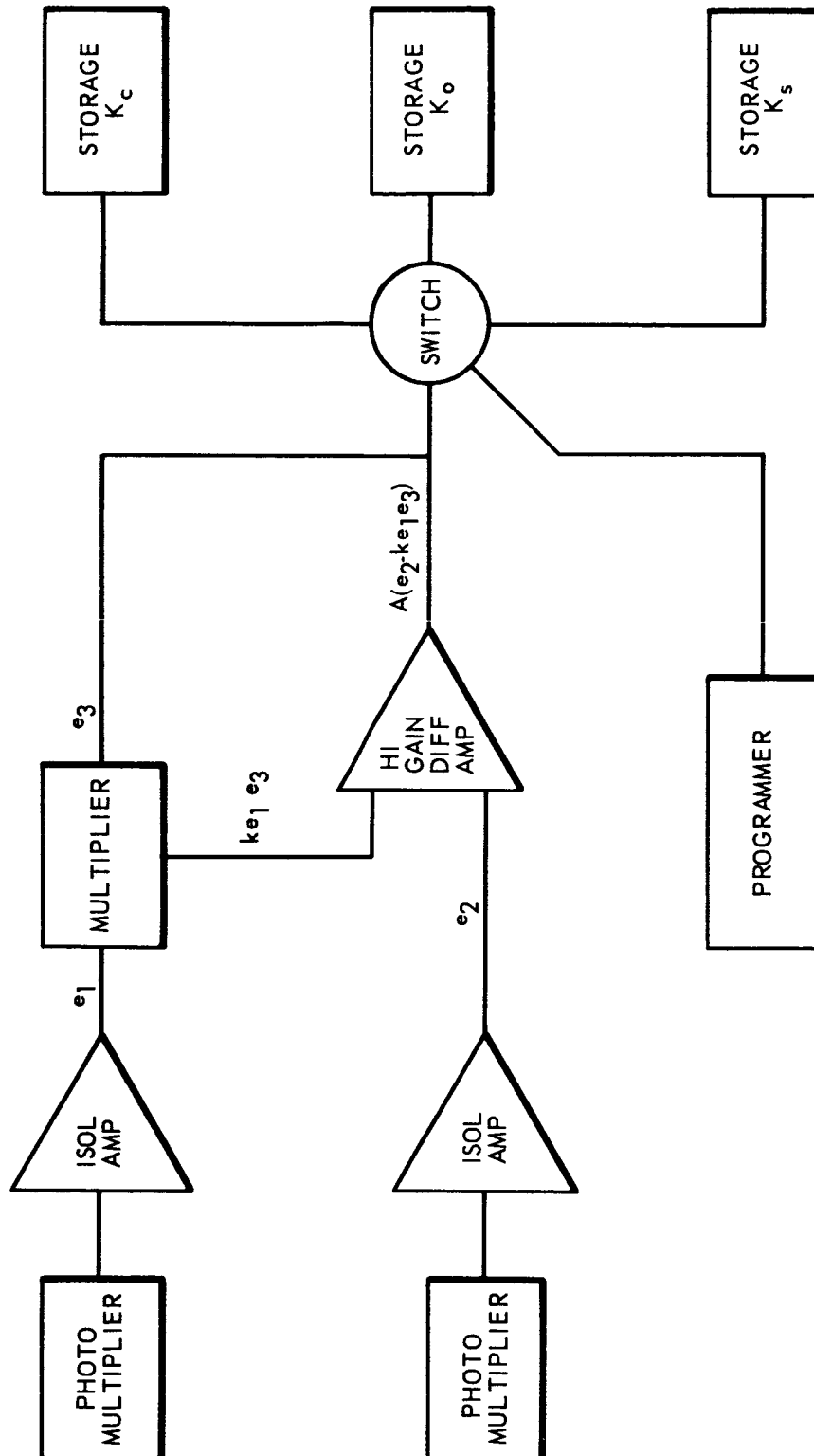


Figure 7. First Ratio Computation and Storage



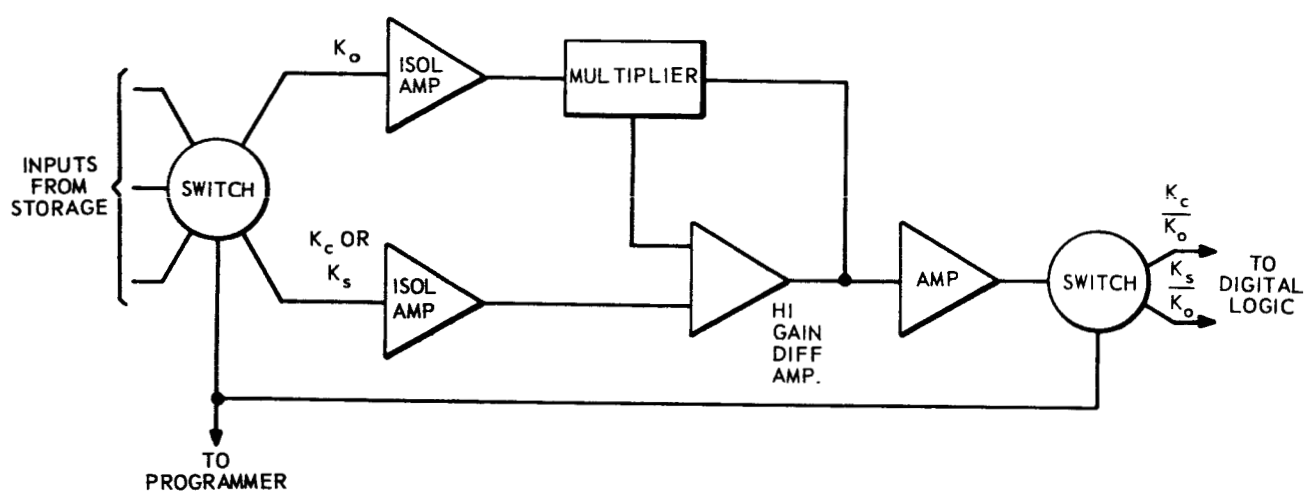


Figure 8. Second Ratio Computation and Amplification

The high-gain differential amplifier to be used is the Philbrick PP-65 type amplifier. It has an open-loop gain of 20,000 and would provide an error in the ratio computations of 5 parts per million. This is of no consequence in the overall accuracy of the computer. A gain of 1000 could be tolerated to retain an accuracy of 0.5% in the ratio computations. The amplifier uses all silicon, solid-state components.

Figure 9 is a block diagram showing the elements of a circuit for yielding an output which is proportional to the ratio of two given voltages. In this circuit the two input voltages are  $e_1$  and  $e_2$ , one is applied to the multiplier and the second is applied to the difference amplifier. An examination of the circuit shows that the output from the amplifier. An examination of the circuit shows that the output from the amplifier, which provides the output and which also furnished one input to the multiplying circuit, is

$$e_3 = A(e_2 - ke_1e_3)$$

If the amplifier provides a high gain, then

$$\frac{e_3}{A} = 0$$

and:

$$e_2 - ke_1e_3 = 0$$

so that finally

$$e_3 = \frac{k'e_2}{e_1}$$

The output will vary for each programmed step. Each such measured ratio will vary if this condition exists, and a valid "answer" for the  $K_s/K_o$  ratio is then awaited. The Schmitt triggers are set up to fire as follows: trigger 1 fires at 12.2 volts; trigger 2 fires at 11.8 volts; trigger 3 fires at 0.5 volts;

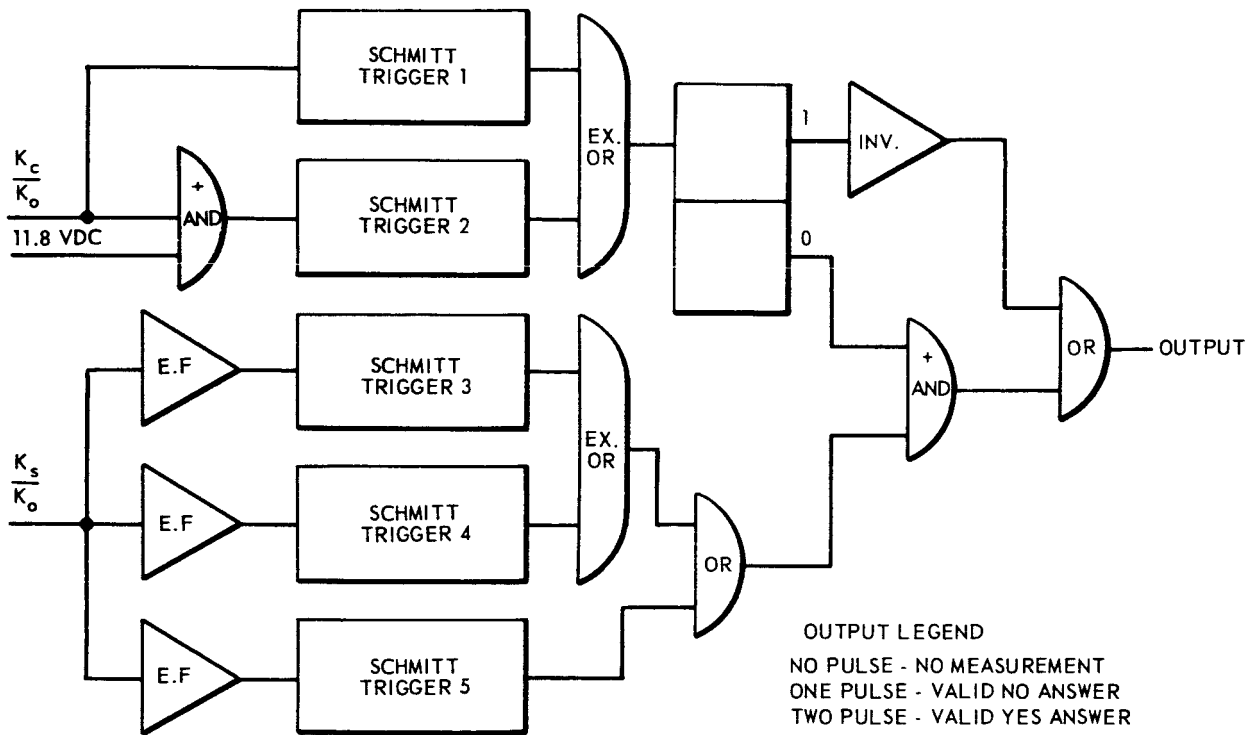


Figure 9. Digital Logic

trigger 4 fires at 9.9 volts; and trigger 5 fires at 10.1 volts. Under these conditions the logic follows: if the  $K_c/K_o$  ratio is less than 11.8 volts, no output pulse will be obtained since trigger 1 and trigger 2 will remain off, and the flip-flop output to the AND gate will remain down, thus inhibiting a pulse that may result from the  $K_s/K_o$  ratio. For  $K_c/K_o$  between 11.8 and 12.2 volts (less than  $\pm 2\%$  of 12.0 volts), trigger 2 will be fired. The signal is then passed through the exclusive OR gate and reverse the state of the flip-flop. The signal from the flip-flop is inverted and passed on to an OSMV (not shown) which provides an output pulse. If the  $K_c/K_o$  ratio is greater than 12.2 volts, trigger 1 and 2 both fire, and the triggered signals are inhibited by the action of the exclusive OR gate; and again no output pulse is obtained.

Assuming that the  $K_c/K_o$  ratio is between the desired limits, then the logic for the  $K_s/K_o$  ratio follows. The third trigger is adjusted to fire for almost any value above the noise level, therefore, if  $K_s/K_o$  is less than 9.9 volts, an output pulse will be obtained, indicating a positive rotation of the polarized light. If the  $K_s/K_o$  ratio lies between 9.9 and 10.1 volts ( $\pm 1\%$  of 10 volts), triggers 3 and 4 will fire, and trigger 5 will remain off. The output signal from the triggers will be inhibited by the exclusive OR gate, and therefore no output pulse will be obtained, thus indicating no rotation of the polarized light because of the sample. For  $K_s/K_o$  ratios greater than 10.1 volts, trigger 5 will fire and provide an output pulse to indicate a positive answer. Therefore, the output pulses obtained will indicate first by no pulse that the system is not operational or has failed in some manner. One pulse will indicate the system is functioning properly, but the sample under test has not provided a rotation of more than  $2\%$ . A two-pulse output will indicate the system is functioning properly, and a rotation of the polarized

light through the sample under test of more than 2% has occurred.

Power requirements for the electronics just described are about 0.8 watts. However, the energy requirement can be reduced by programming the power properly. Power only need be supplied to the photomultiplier tube, the first ratio computer circuits, and the storage circuits for the initial phase. Then the power is removed from all but the storage circuits and is applied to the remainder of the circuits for the final phase of computation. The power requirement is reduced in this manner so that the power requirements for the initial phase is about 0.7 watts, and the requirements for the second phase is 0.2 watts. However, the time requirement in the second phase can be held to about 0.5 seconds.

### 2.3.3 Conclusions

The data presented indicates that the theoretical development of the electronic simple ratio for obtaining optical rotatory dispersion measurements is valid. Common instrumental problem areas such as the unequal transmission of the Glan polarizing prisms, which render the Rouy-type polarimeter a qualitative instrument, do not affect the optical rotation measurements by the simple ratio technique.

The use of the development has led to a design of a polarimeter to detect extraterrestrial life, by measuring the optical activity in soil.

### 3. PROCESSING OF SOILS

#### 3.1 Background

The measurement of optical activity in soil presents several serious problems. Intense scattering of plane polarized light from solid particles would be encountered which would completely destroy any chance of detecting a signal. In addition, the concentration of biological materials in soils is quite low when compared to the concentration of inorganic substances. Therefore, various approaches to obtaining extracts from soil were investigated with the object in mind of concentrating the optically active components present. This extract could then be filtered to eliminate the insoluble impurities (scattering centers).

#### 3.2 Results

##### 3.2.1 Evaluation of Soil Extracts

Extracts of soils were prepared with water, 0.15 N HCL, 0.15 N NaOH and 0.5 N NaOH solution. These solutions were mixed with the individual soil samples for sufficient lengths of time to insure saturation of the extracting agents. All extracts were filtered through a 0.45 micron Millipore filter. Representative concentrations of dissolved components in the water, acid, and base extracts of the soil were found to be 0.65 mg/ml, 11.4 mg/ml, and 7.7 mg/ml, respectively.

For optical rotation measurements, water-soluble extracts were used without further dilution. The acid-soluble extracts required a dilution of 1:2, and the base soluble extract required a dilution of 1:100. Using the simple ratio, optical rotatory dispersion measurements of these extracts were obtained. Results for the water, acid, and base extracts are shown

in figure 10, figure 11, and figure 12, respectively. From an examination of these figures, optically active components can be seen to be present. However, calculation of the specific rotation from the angular rotations of the aqueous acid and basic extracts at 254 mu show both the water and 0.15 N NaOH extract to have the highest specific rotations. The 254 mu wavelength was selected since the emission of the mercury lamp discussed in section 2.3.2.2 gives an extremely intense peak at 253.7 mu.

In an attempt to identify the optically active components in the alkaline extract of soil, two alkaline extracts (0.15 N NaOH and 0.5 N NaOH) were prepared. Recently, Anderson (Soil Science, 80, 169 (1958) and Soil Science, 91, 156 (1961) had pointed out that nucleic acids present in soils may be found in the alkaline extract. Therefore, the use of the 0.5 N extract could lead to the formation of mononucleotides by hydrolysis of any RNA present in soil; whereas the RNA in 0.15 N NaOH would not be hydrolyzed. This is based upon the fact that Boulanger and Montreuil (J. Bull, Soc. Chem. Biol., 33, 784 (1951) needed a 0.5 N NaOH solution to hydrolyze RNA. Thus, alkaline hydrolysis of the RNA to its mononucleotides would be expected to exhibit more positive angular rotations than solutions containing unhydrolyzed RNA since adenosine (see figure 13) exhibits significant positive angular rotations in the 230 to 255 mu region. However, comparison of the optical dispersion curves (in figure 12) obtained on the two base extracts in this wavelength region indicated that the optical activity of the more basic extract exhibited negative angular rotations, and the other basic extract exhibited positive angular rotations.

Since the results on the alkaline extracts of soil were contrary to what was expected, a study of the effect of sodium hydroxide on the optical

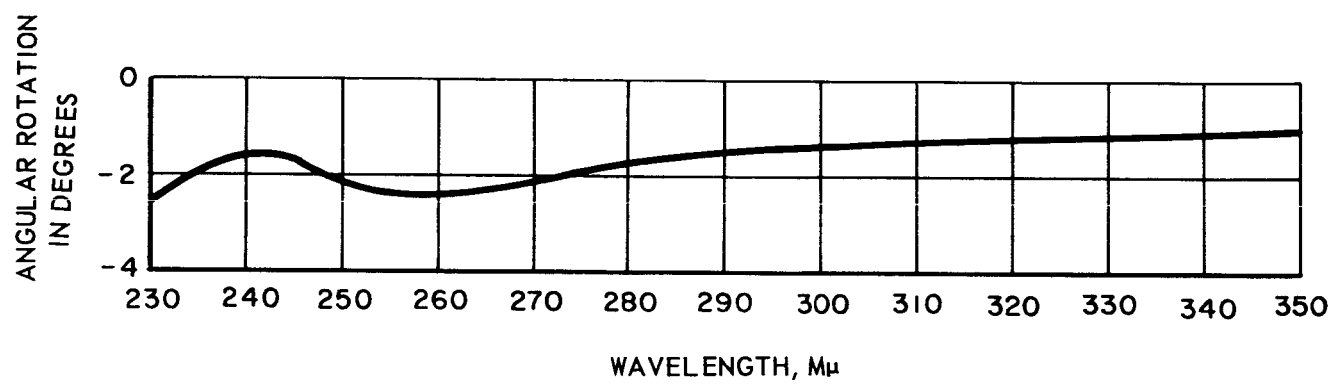


Figure 10. Optical Rotatory Dispersion Spectra of Clear and Cloudy Water-Soluble Extract of Soil (Concentration 0.65 mg/ml)



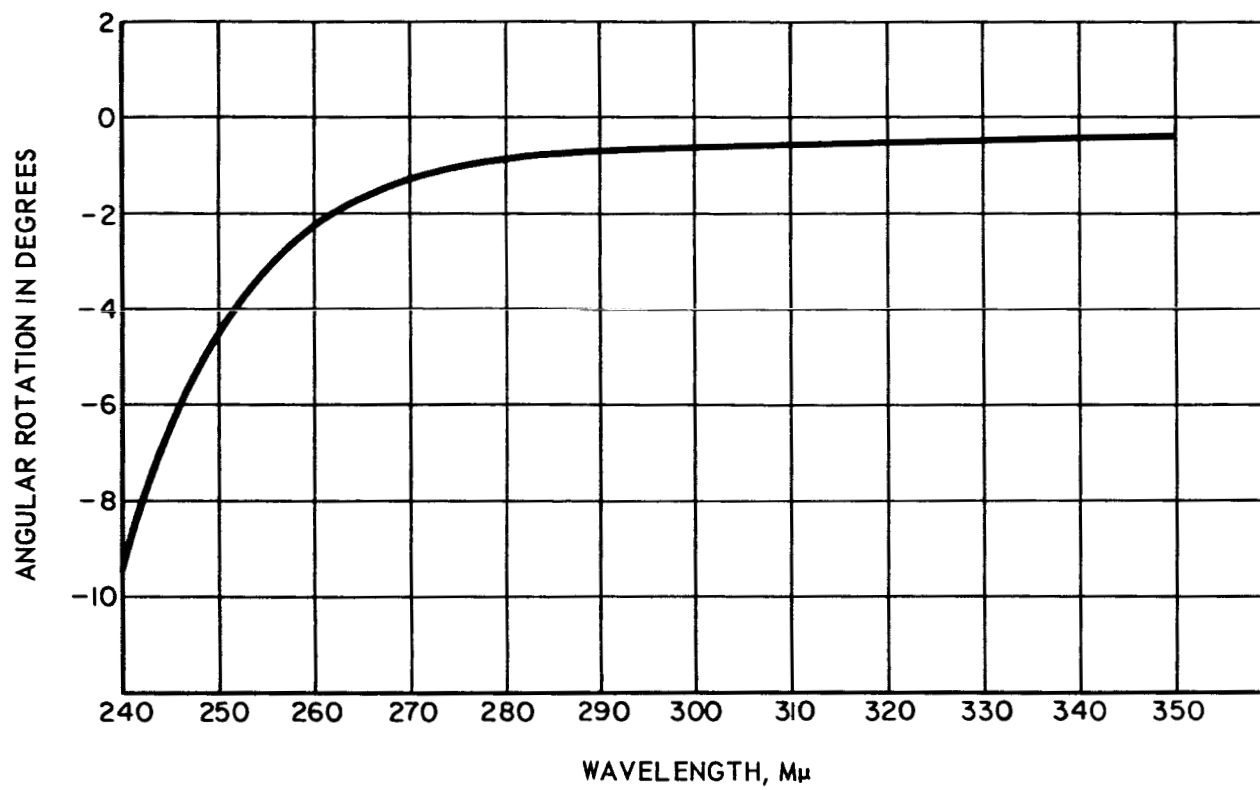


Figure 11. Optical Rotatory Dispersion Spectra of Acid-Soluble Extract of Soil (Concentration 5.7 mg/ml)

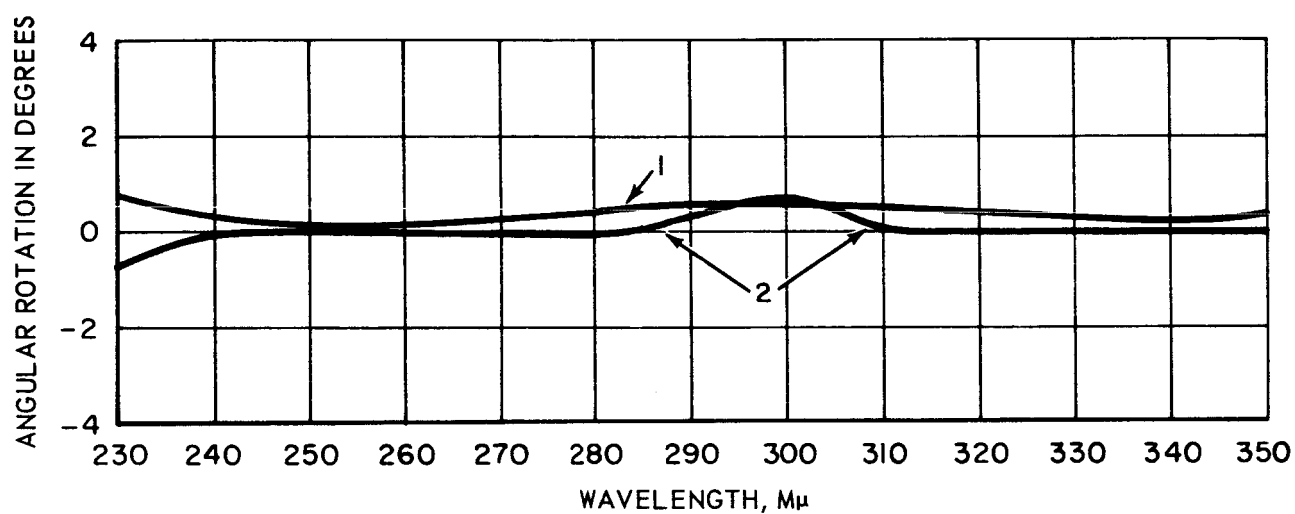


Figure 12. Optical Rotatory Dispersion Spectra of Base-Soluble Extracts of Soil (Concentration 0.077 mg/ml: (1) 0.15N NaOH Extract and (2) 0.5N NaOH Extract)

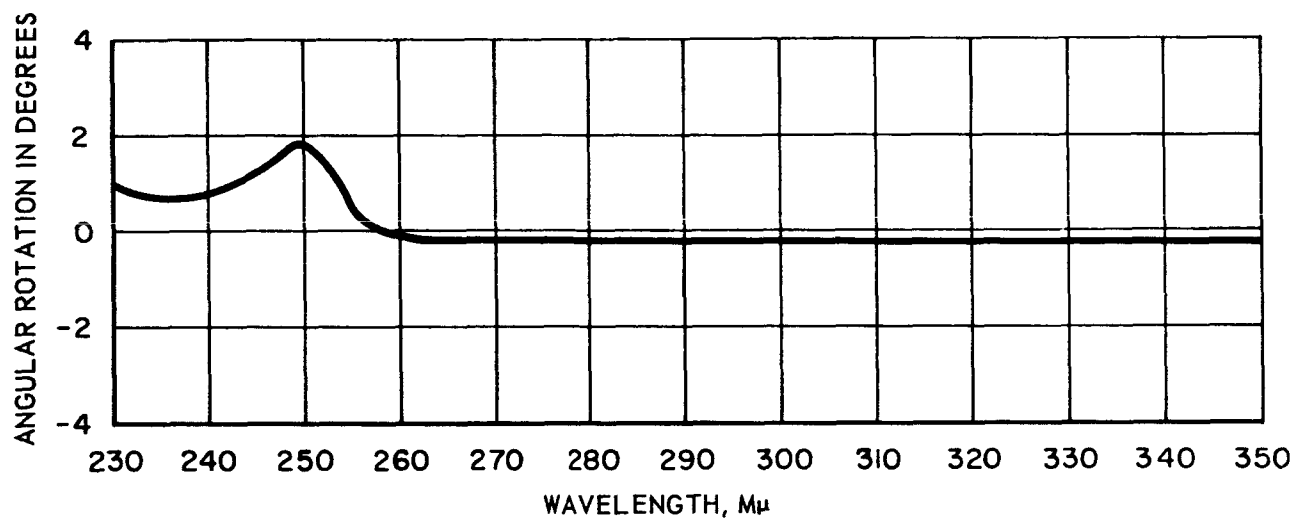


Figure 13. Optical Rotatory Dispersion Spectra of Adenosine (Concentration 0.021 mg/ml)

rotatory dispersion curves of RNA was initiated. Three solutions of RNA were prepared, a solution buffered at pH 4.5, a 0.15 N NaOH solution, and a 0.5 N NaOH solution. The results of the dispersion measurements on these solutions are shown in figure 14. It is of interest to note from the figure that the RNA which has been dissolved in the buffered solution exhibits negative angular rotation in the wavelength region from 230 to 300 mμ. There is the appearance of a Cotton curve between 250 and 275 mμ. The maximum portion of the curve occurs at 265 mμ and the minimum at 255 mμ. Upon dissolving the RNA in both 0.15 N NaOH and 0.5 N NaOH, a shift in the maximum for both basic solutions occurs. That is from 265 to 262 mμ for the 0.15 N NaOH solution and to 260 mμ for the 0.5 N NaOH solution. Both minimums are shifted from 255 to 245 mμ. Also, for the case of the buffered solution the maximum has a negative angular rotation, whereas the basic solutions now exhibit positive rotations. Adenosine (see figure 13) also has a positive angular rotation associated with its maximum. Thus, optical rotatory dispersion measurements on the hydrolysis products of RNA should give a curve similar to the dispersion curve of the mononucleotides. In addition, a comparison of the dispersion curves for both basic solution reveals that in the region from 230 to 250 mμ, the 0.5 N NaOH solution has larger negative angular rotations. The reason for this behavior is not clear at the present time. In the case of the alkaline extracts of soil, the 0.15 N NaOH extract showed positive angular rotations between 230 and 250 mμ, whereas the 0.5 N NaOH showed negative angular rotations.

Since the presence of proteins, enzymes, etc. in soil cannot be discounted, the effect of acid and basic pH on the rotatory dispersion

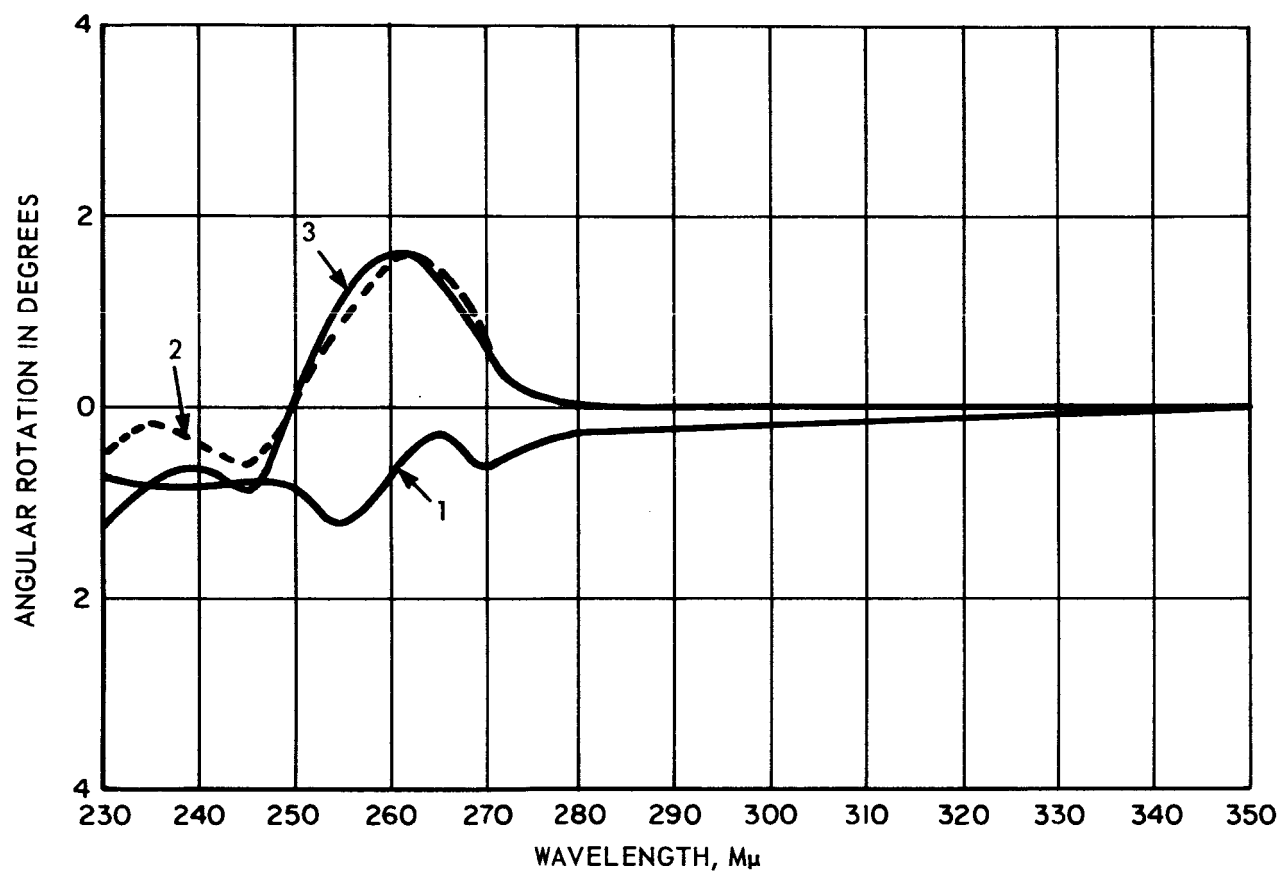


Figure 14. Optical Rotatory Dispersion Spectra of Purified RNA from Yeast: (1) in pH4.5 Buffer, at 0.01%, (2) in 0.15N NaOH, at 0.01%, and (3) in 0.5N NaOH, at 0.01%

curves of such substances was also investigated. The horse serum was decided upon since it is generally considered to be a complex mixture of the above substances. The result of the addition of acid on the dispersion curve of horse serum is shown in figure 15. It may readily be seen that the dispersion curves for the horse serum dissolved in 0.9% KCl and 0.15 N HCl inhibit normal dispersion curves, that is, no Cotton regions are observed. However, the acid solution reveals larger negative rotations than the salt solution between 240 and 300 mμ. Proteins in strongly acid or basic media undergo denaturation with an accompanying increase in optical activity toward negative angular rotations.

The effect of basic pH as well as sodium ion concentration on the rotatory dispersion curves of horse serum are shown in figure 16. It may readily be seen from this figure that the horse serum dissolved in both 0.15 N NaOH and 0.5 N NaOH exhibits complex dispersion curves. There appears to be two Cotton regions, with the associated maximums occurring at 290 and 308 mμ and with the minima occurring at 295 and 320 mμ. Both alkaline solutions exhibited higher negative angular rotations than the salt solution. This observation suggests molecular conformational changes within the horse serum because of the presence of NaOH. Also, the solution containing the lowest amount of sodium ion appears to exhibit larger negative rotations than the solution containing the higher concentration of Na ions. Thus, it may be possible that the sodium ion may in some way inhibit conformational changes within the horse serum.

If any nucleic acid such as RNA is present in soil, it would be expected to be found in the presence of other substances such as protein,

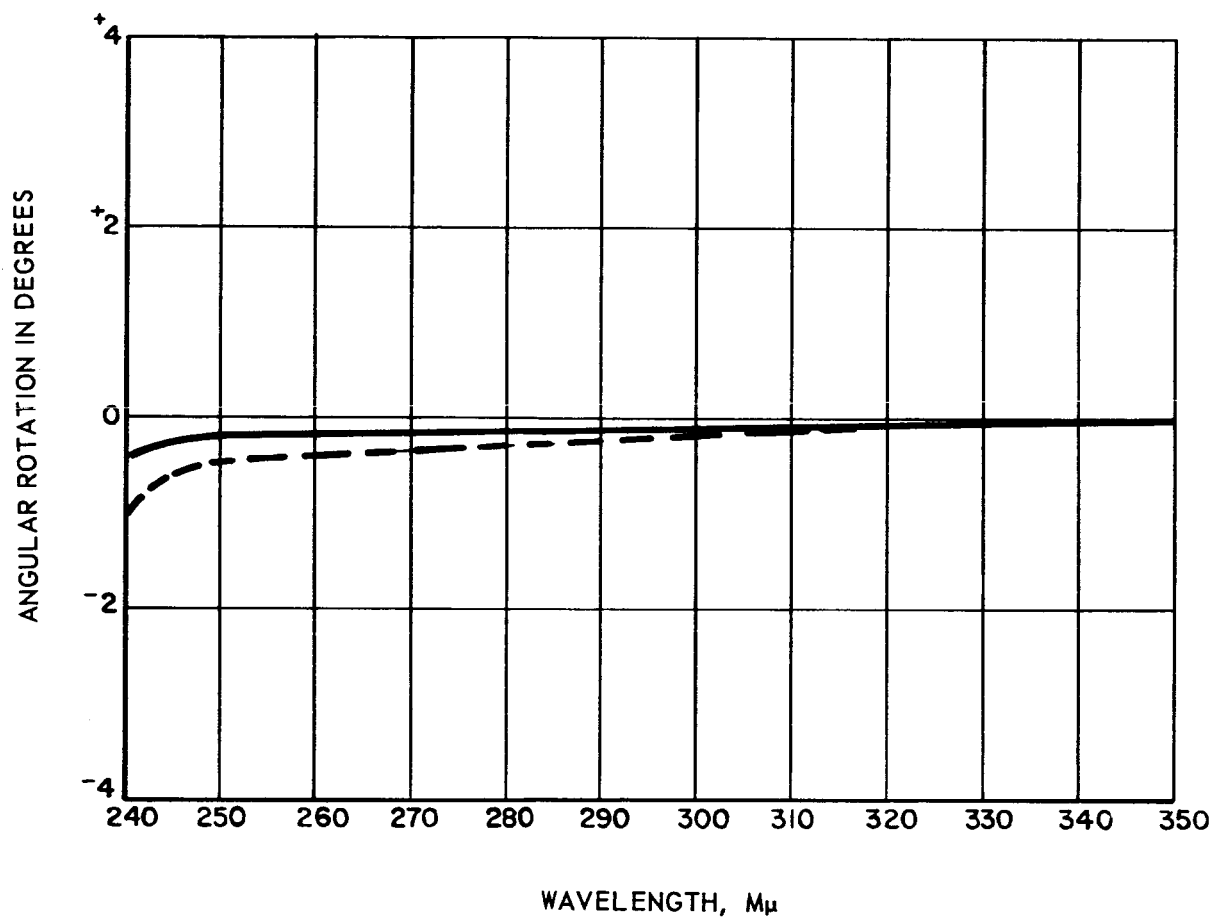


Figure 15. Optical Rotatory Dispersion Spectra of Dialyzed Horse Serum  
Solid Line, in 0.9% KCl at 0.16% and Dotted Line, 0.15 NHC1  
at 0.16%

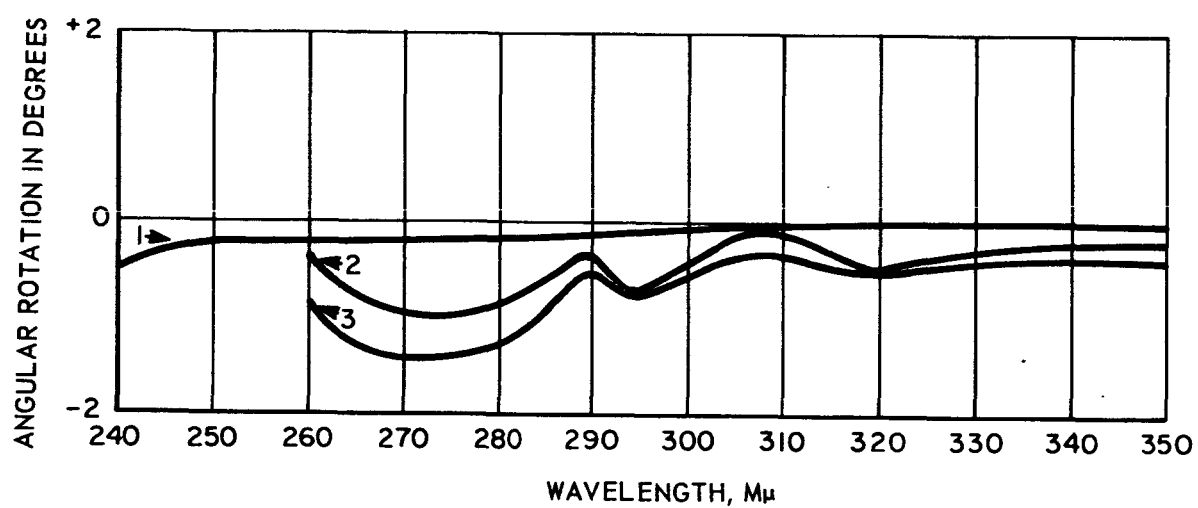


Figure 16. Optical Rotatory Dispersion Spectra of Dialyzed Horse Serum in: (1) 0.9% KCl, at 0.16% (2) 0.5N NaOH, at 0.16%, and (3) 0.15N NaOH, at 0.16%



etc. Thus, it was of interest to obtain the optical rotatory dispersion curves on a mechanical mixture containing RNA and horse serum in alkaline solution. The dispersion curves in figure 17 represent measurements on a mechanical mixture of horse serum and RNA in 0.15 N NaOH and 0.5 N NaOH. It may be seen from an examination of this figure that the alkaline solutions containing the lower concentration of sodium ion shows a Cotton region between 270 and 300 mu. The maximum associated with this region appears to be at 290 mu and the minimum at about 278 mu. The solution containing the higher concentration of Na ion revealed no Cotton region in the portion of the spectrum examined. Also, in the region from 255 to 285 mu, the mechanical mixture dissolved in 0.15 N NaOH exhibited larger negative angular rotations than the 0.5 N NaOH solution. Thus, the effect of sodium ion inhibition toward molecular conformational changes within the horse serum, must also hold for mechanical mixtures.

It is apparent from the results that not only are strong NaOH solutions capable of hydrolyzing RNA to their mononucleotides, but also that the sodium ions in sufficient concentrations may inhibit conformational changes in such substance as are found in horse serum. Thus, in the basic extract of soils, it appears safe to assume that these effects may also occur. Both hydrolysis reactions, as well as molecular conformational changes (such as denaturation) of high molecular weight substances, appear to enhance the optical activity. Thus, at the present time, the use of 0.15 N NaOH for preparation of a soil extract appears to be the best approach for the detection of optical activity in soils.

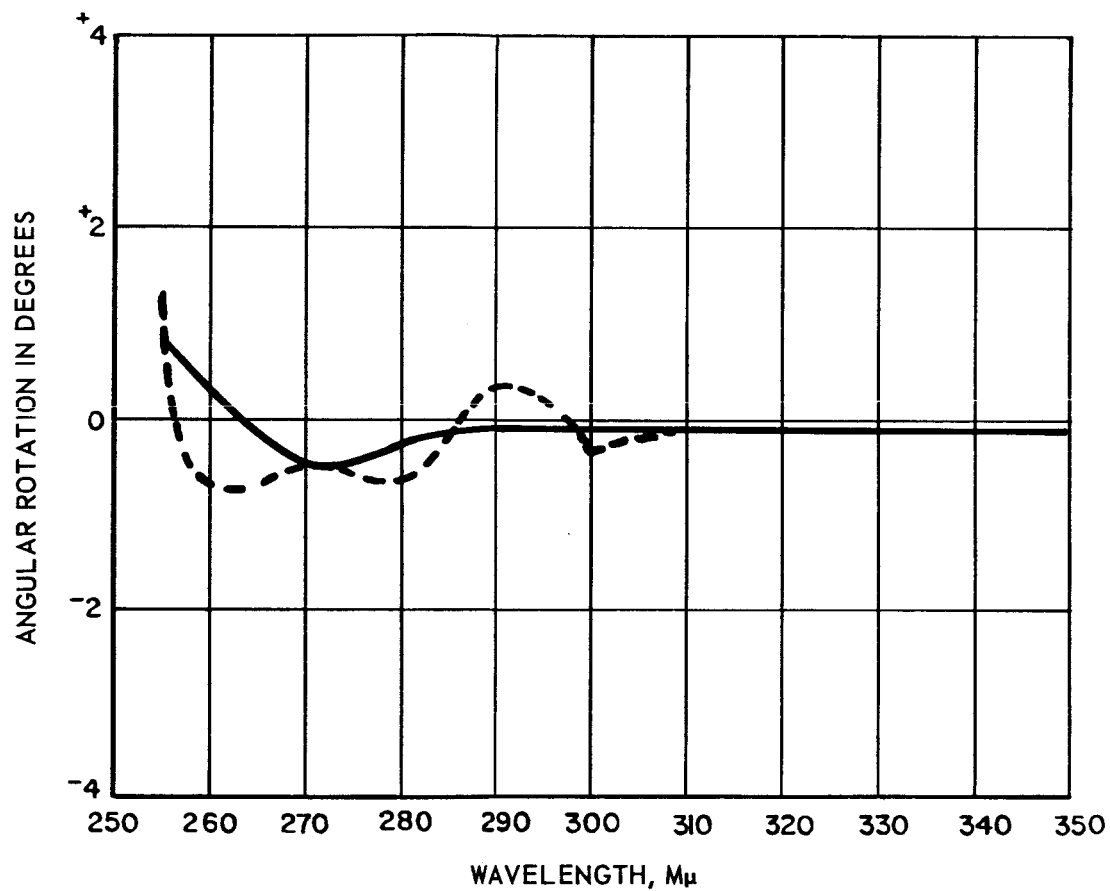


Figure 17. Optical Rotatory Dispersion Spectra of Mixtures of RNA, at 0.005% and Horse Serum, at 0.08% in Solid Line, 0.5N NaOH and Dotted Line 0.15N NaOH

### 3.2.2 Instrumental Design for the Sampling System of a Flight-Prototype Polarimeter

Based upon the results of the evaluation of soil extracts, an extraction system for soil using 0.15 N NaOH has been designed for the flight-prototype polarimeter. A schematic of this system is shown in figure 18. This system consists of a small sealed reservoir to contain the extraction solvent, a pneumatically driven double-end reciprocating pump with slide valve and check valves, filters for removal of particulate matter, and the sample cuvette.

The soil sample may be delivered to the sample collection chamber in a dry powder form, as an airborne aerosol or as a soft slug. At the designated sequence step in the instrument program, the inlet and outlet parts of the sample chamber are sealed off with squib valves, and the solvent is injected and circulated through the sample chamber by the pump (see figure 19) until the solution light transmission is reduced to a predetermined level. The pump is then stopped and the analysis made. If this level is not reached within a specified time, the programmer performs the shutdown function.

### 3.3 Conclusions

The use of 0.15 N NaOH for preparation of a soil extract appears to be, at present, the best approach for the detection of optical activity in soil. Optical activity has been observed in an alkaline extract containing only 0.08 mg/ml of dissolved components. However, these optically active components have yet to be identified. Therefore, correlation of the optical activity with the dissolved components in the alkaline extract

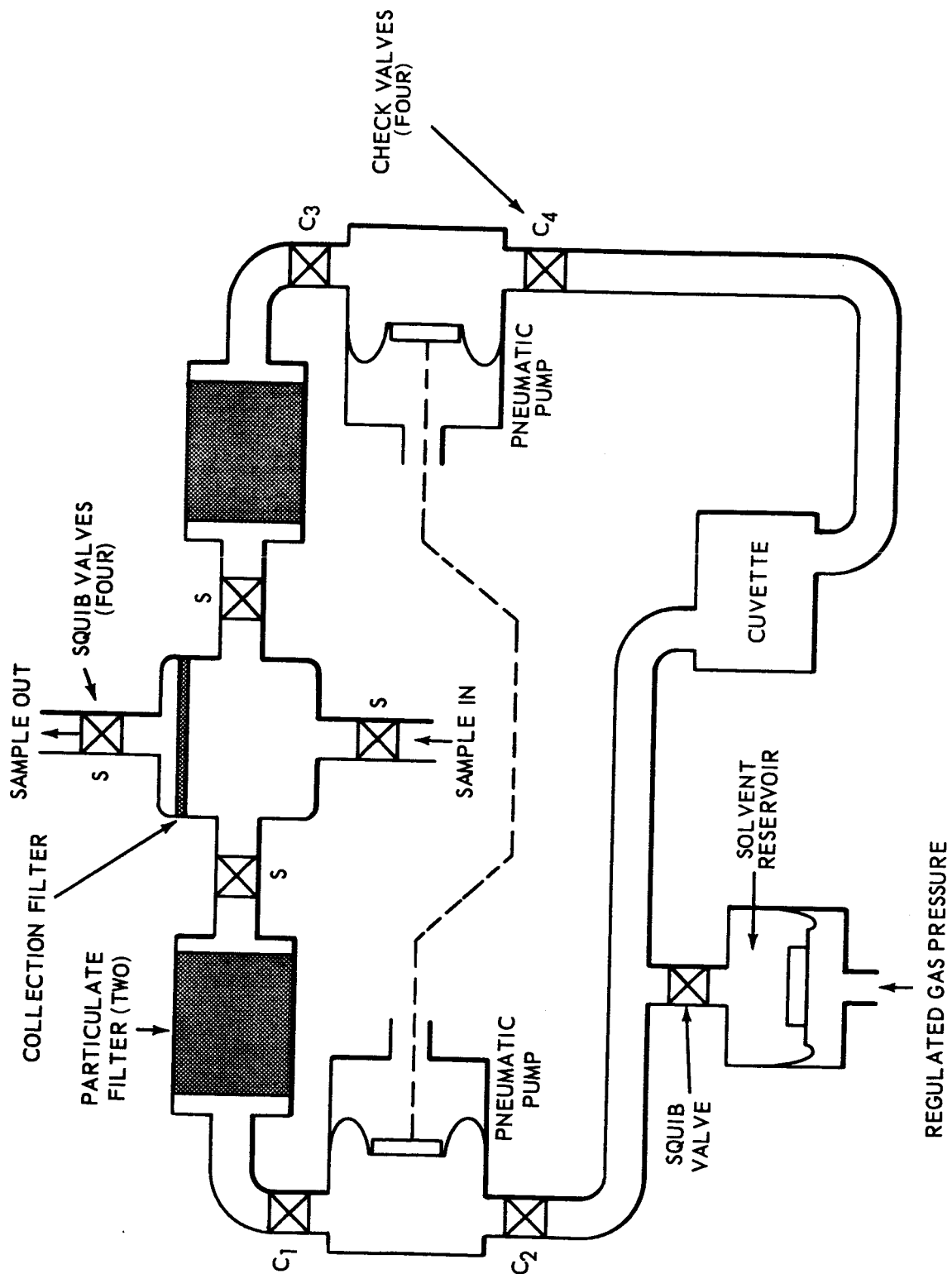


Figure 18. Sample Process System

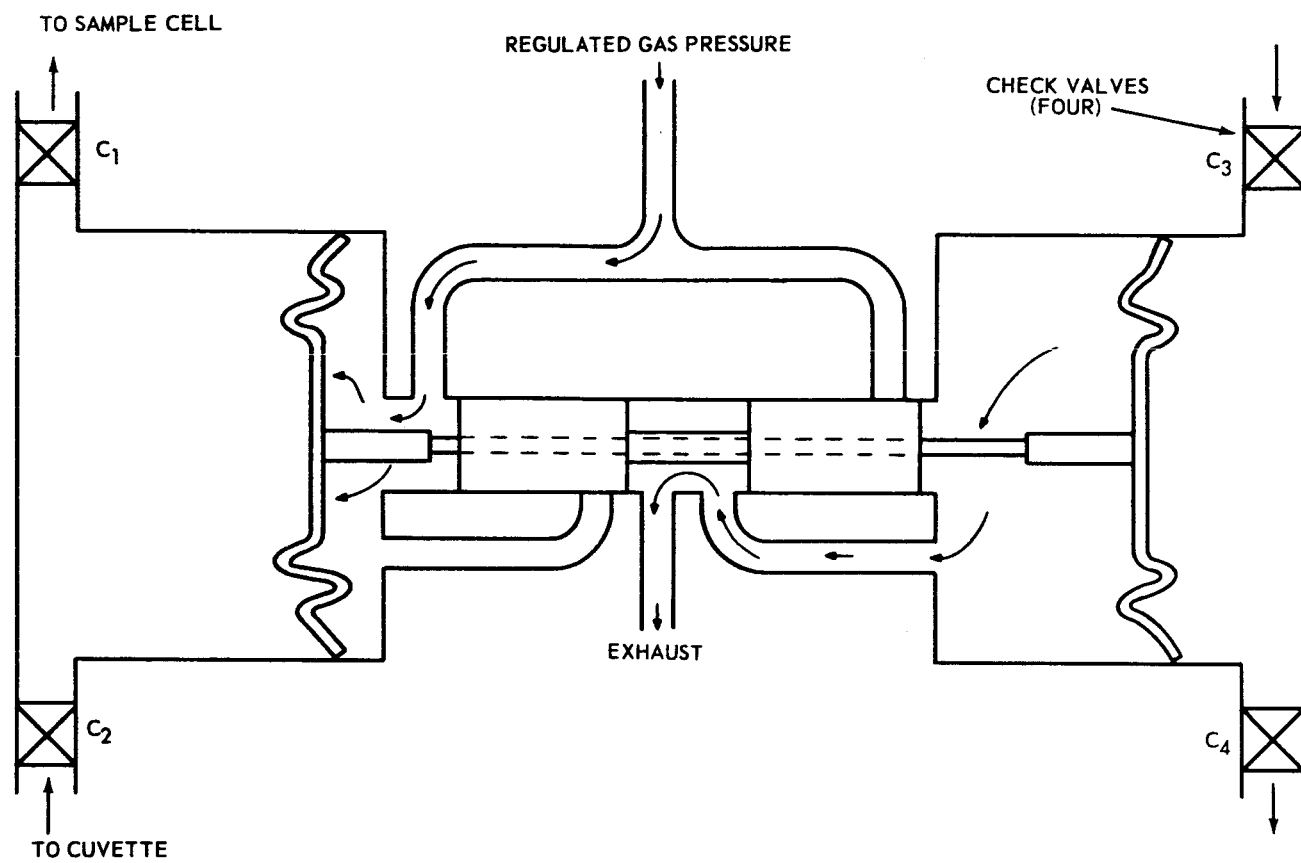


Figure 19. Pneumatic Recirculating Pump

of soil has not been made. On the basis of these studies, an extraction system has been designed to be used in conjunction with the flight-prototype polarimeter.

#### 4. SUMMARY

Melpar has developed two new experimental procedures for measuring optical activity in absorbing and scattering media. These procedures are defined as the ration of the difference to a constant and a modified form, the simple ratio. The theoretical development of the simple ratio has been proven valid by experimentation. This ratio is not effected by the instrumental problems which render the Rouy polarimeter a qualitative instrument. The simple electronic ratio readily lends itself to use in unmanned experimental probes, since it requires no continuously moving parts. A polarimeter, based upon this ratio, has been designed to detect optical activity in soils.

An extraction procedure has been developed which permits the detection of the optical activity in 0.08 mg/ml of dissolved soil components. Based upon this procedure, an extraction processing system has been designed to be used in conjunction with the polarimeter.

## 5. PROPOSED FUTURE PROGRAM

a. The soils used in this investigation have been obtained in the vicinity of Melpar's laboratories. In order to generalize the results of this program, soils obtained from a variety of sources must be examined.

b. To establish the results of this investigation on a quantitative basis, the optically active components in the alkaline extract of soils must be identified and correlated with the observed optical rotations of the extract.

c. An instrument must be developed which is capable of quantitatively analyzing the extract of soils to determine what biological components are present. This means providing the present design with some means to monochromate light of at least three wavelengths and providing a method of monitoring those wavelengths.